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Department of Parasitology, Polish Academy of Sciences
Head: Prof. Dr. Witold Stefański, Member of P.A.S.

Bożena GRABDA

Pleurogenes intermedius Isaitchikoff, 1926 and *Prosotocus mirabilis* sp. n. *(Trematoda, Lecithodendriidae)* in Poland

Pleurogenes intermedius Isaitchikoff, 1926 i *Prosotocus mirabilis* sp. n. *(Trematoda, Lecithodendriidae)* w Polsce

When examining *Rana terrestris* coming from the Łuże Marshes in the Forest Kampinos in 1954 we found cysts of connective tissue inside which there were single mature flukes. The cysts were found in the abdominal wall of two frogs.

Further investigations carried in 1955—57 showed that these flukes occurred exclusively in cysts of connective tissue and were pretty frequent in that territory. We determined them as *Pleurogenes intermedius* Isaitchikoff, 1926. In the course of further examination of *Pl. intermedius* we found another species which had not been described before and we called it *Prosotocus mirabilis* sp. n. It occurred in the wall of the frog stomach also in cysts of connective tissue.

The phenomenon of the transition of typical intestinal forms such as flukes of the subfamily of *Pleurogenetinae* to the body cavity has so far been known only in the case of one species, *Mehraorchis ranarum* Srivastava, 1934, occurring in cysts in the body cavity of *Rana cyanophlyctis*. We think it advisable to give a detailed description of the species found by us as well as to characterize their habitat since they occur on a small and fairly isolated territory.

We wish to thank here Professor R. Ph. Dollfus for his valuable help in my work on these species.

The Forest of Kampinos now occupies the territory north-west of Warsaw. The Vistula forms its northern and the Bzura its western borders, while its southern boundary is the high Vistula terrace. That territory lies on two terraces, one being a former flood plain and the other a dune land. There are three dune belts and wide marsh belts

between them on the latter, which constitutes four fifths of the whole area of the Forest. There are also smaller marshes in the hollowings between the dunes. These are mostly low peat bogs mostly overgrown by sword-grass.

The Łuże Marshes, from which came the examined frogs lie in the extreme west part of the Forest near Warsaw. As regards the lay of the land they are clearly isolated from the rest of the Forest. They are confined by high dunes in the north and west and the high Vistula terrace in te south. A watershed runs through the surrounding dunes which directly separates the Vistula basin, to which the Łuże Marshes belong, from that of the Bzura, which comprises the rest of the Forest.

There are a few small bodies of water in the Łuże Marshes most of which dry up in summer. In spring a surplus of water is removed directly into the Vistula by a drain which has supplanted a rivulet.

The water sheets are surrounded with reeds made up of *Scirpus tabaernemontani* and *Phragmites communis* as well as *Potamogeton natans* and *Chara fragilis* farther in. The shallower places are overgrown by *Carex stricta,* which forms characteristic columns passing into a sourmmeadow in the peripheries.*)

Of amphibians, *Rana terrestris* Andrzejewski (syn. *R. arvalis* Nils.) were most frequent here as throughout the Forest and there also occurred *Rana temporaria* L., *R. esculenta* L., *Bombina bombina* L., *Bufo bufo* L., *B. calamita* Laur., *Hyla arborea* L., *Triturus vulgaris* L., but only *R. terrestris, R. temporaria* and *R. esculenta* were examined.

In addition to *Pl. intermedius* and *P. mirabilis* sp. n. we found the following fluke species in *R. terrestris* on that territory: *Pleurogenes claviger* (Rudolphi, 1819), *Pleurogenoides medians* (Olsson, 1876), *Prosotocus confusus* (Looss, 1894), *Brandesia turgida* (Brandes, 1888), *Opisthioglyphe ranae* (Frölich, 1791), *Haplometra cylindracea* (Zeder, 1800), *Haematoloechus variegatus* (Rudolphi, 1819), *Haematoloechus asper* (Looss, 1899), *Diplodiscus subclavatus* (Pallas, 1760), *Gorgodera cygnoides* (Zeder, 1800), *Gorgoderina vitelliloba* (Olsson, 1876), *Tetracotyle cristallina* (Rudolphi,

* The data on the Forest of Kampinos come from a study by R. Kobendza: Stosunki fitosocjologiczne Puszczy Kampinoskiej [Phytosociological Relations in the Forest of Kampinos]. Planta Polonica, Vol. II, Warszawa, 1930.

1819), mesocercaria *Alaria alata* (Goeze, 1872), *Polystomum integerrimum* (Frölich, 1791).

In *R. esculenta* occurred *Pl. claviger, Pl. medians, P. confusus, O. ranae, H. variegatus, H. asper, D. subclavatus, G. cygnoides.*

The occurrence of a considerable number of fluke species in a relatively small area is a characteristic feature of Łuże. The extensity and intensity of invasion however are very low. In this respect too, Łuże differs from the rest of the Forest and also from other areas round Warsaw. *Pl. intermedius* was the species of the highest extensity, the remaining species being sometimes less frequent than *P. mirabilis.*

Pleurogenes intermedius Isaitchikoff, 1926

This species, first described by Isaitchikoff in 1926, was found in the small intestine of *Rana arvalis altaica* Kaschtchenko, coming from the vicinity of Omsk, Siberia. So far this has been the only place notorious for the occurrence of these flukes.

The flukes we found at Łuże (Fig. 1) show considerable differences on comparison with those described by Isaitchikoff. They consist first of all in localization in the host and a certain difference in structure resulting from this. All flukes found at Łuże occurred exclusively in cysts of connective tissue in the lower part of the abdominal cavity, usually between the peritoneum and the bladder wall or between the peritoneum and the abdominal muscles close to the bladder. The cysts, irregular in shape, flat, up to 1 cm in diameter, contained 1—8 flukes each, usually placed in a few chambers connected with one another by narrower canals (Fig. 2). Up to nine flukes were found in one frog. The intestinal forms described by Isaitchikoff were strongly flattened dorso-ventrally and those from the body cavity were relatively thick, their cross-sections were in the shape of an ellipsis approximating a circle. In addition, their mobility and adhesion power were considerably diminished. A substantial increase in body size was also noticeable.

They are large and egg-shaped flukes. Their body is pink in colour and opaque; their yellowish-brown eggs show across it; the cuticle is thickly covered with spines. The oral sucker is situated terminally; the ventral sucker, far less developed, lies in the front of the body at one third of body length.

The alimentary tract. The oral sucker is immediately followed by a prepharynx, a small spherical pharynx and a short oesophagus.

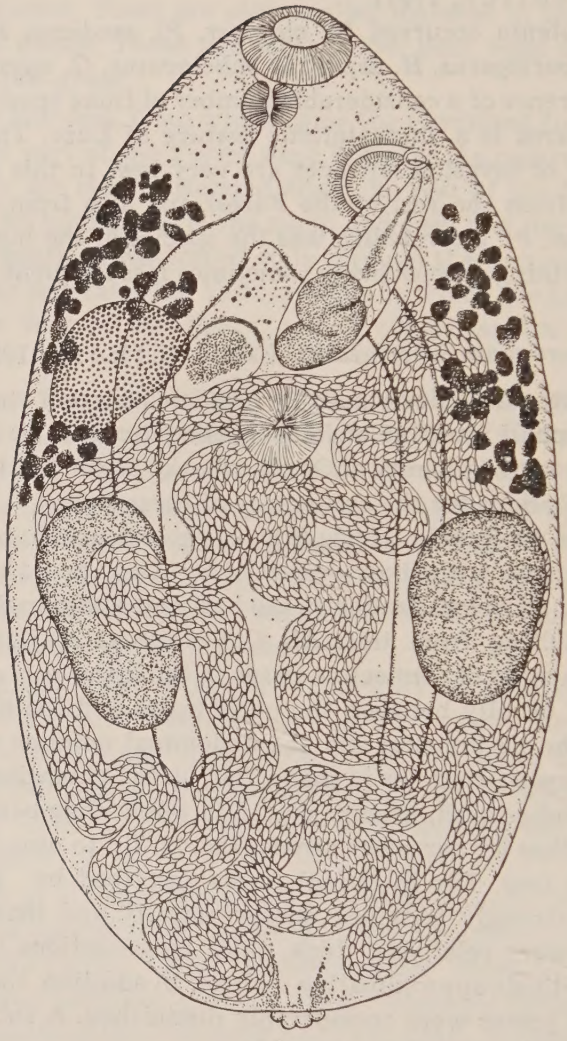

Fig. 1. *Pleurogenes intermedius* Isaitchikoff, 1926

Table I

Frogs examined

Frog species	Number examined	*Pleurogenes intermedius*			*Prosotocus mirabilis*		
		Number invaded	Extensity	Number of flukes found	Number invaded	Extensity	Number of flukes found
R. terrestris	120	38	31.6	134	4	3.3	64
R. temporaria	4	1	—	2	—	—	—
R. esculenta	22	—	—	—	—	—	—

Table II

Measurements of *Pleurogenes intermedius* Isaitchikoff

	after Isaitchikoff	flukes from Łuże
Body length	3.45 — 3.60 mm	2.85 — 4.29 mm
Body width	2.36 — 2.45 „	1.63 — 2.50 „
Oral sucker	0.25 — 0.34 × 0.40 — 0.45 mm	0.17 — 0.47 × 0.31 — 0.49 mm
Pharynx	0.20 — 0.22 × 0.22 „	0.15 — 0.22 × 0.17 — 0.23 „
Ventral sucker	0.35 — 0.37 × 0.28 — 0.40 „	0.22 × 0.37 „
Ovary	0.35 — 0.42 × 0.32 — 0.42 „	0.47 — 0.63 × 0.34 — 0.56 „
Left testis	0.59 — 0.67 × 0.39 — 0.45 „	0.66 — 1.01 × 0.29 — 0.63 „
Right testis	0.62 — 0.76 × 0.34 — 0.42 „	0.61 — 0.94 × 0.31 — 0.68 „
Eggs	0.034— 0.037× 0.017— 0.02 „	0.033— 0.042× 0.015— 0.02 „

Table III

Measurements of *Prosotocus mirabilis* sp. n.

Body length	1.33 — 2.14 mm
Body width	1.33 — 1.79 „
Oral sucker	0.23 — 0.33 × 0.23 — 0.39 mm
Pharynx	0.09 — 0.11 × 0.09 — 0.13 „
Ventral sucker	0.20 — 0.30 × 0.20 — 0.32 „
Ovary	0.30 — 0.45 × 0.31 — 0.42 „
Left testis	0.45 — 0.70 × 0.31 — 0.55 „
Right testis	0.39 — 0.53 × 0.31 — 0.57 „
Eggs	0.027— 0.031× 0.012— 0.015 „

The intestine bifurcates half way between the suckers. Its branches do not reach the end of the body and terminate a little behind the lower margin of the testes.

The excretory system. The excretory vesicle, type V, with lateral branches closely approaching each other. The terminal part of the bladder may be turned inside out and it then forms a fold around the excretory pore (Fig. 3).

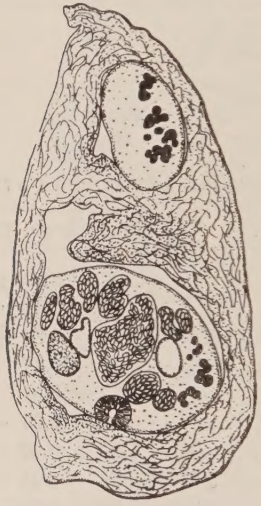

Fig. 2. Cyst containing 2 specimens of *Pleurogenes intermedius*. Section.

Fig. 3. *Pleurogenes intermedius* — Sagittal section.

The genital system. Two oval or less regular testes lie in the middle part of the body or somewhat behind, between the margin of the body and the intestinal branches. A large cirrus sac contains a large coiled seminal vesicle, an ejaculatory duct and a pars prostatica. It empties into the atrium genitale lying somewhat dorsally on the left side of the body at the height of the pharynx. The cirrus is short and like a papilla in shape. Specimens with an extruded cirrus or copulating were found a few times. A kidney-shaped ovary lies over the right testis closer to the dorsal side. Next to it lies a large seminal receptacle, usually filled up with spermatozoa, and an ootype.

Laurer's canal in pretty long and runs down obliquely towards the dorsal surface. Tree-like vitelline glands lies at the sides of the body over the testes. The uterus forms two strongly folded loops in the hind part of the body. The uterine loops in older flukes get straightened up and enlarged, particularly in the middle part of the body, as a result of their filling up with eggs. The terminal part of the uterus forms a strongly muscles S-shaped metraterm emptying into the atrium genitale near the cirrus sac. There are a number of small yellowish-brown eggs covered with a gelatinous envelope which swells considerably in water. Completely developed miracidia are inside mature eggs. Eggs were found in the capillary vessels of the cysts. This shows how they get through the blood system into the bladder and then outside.

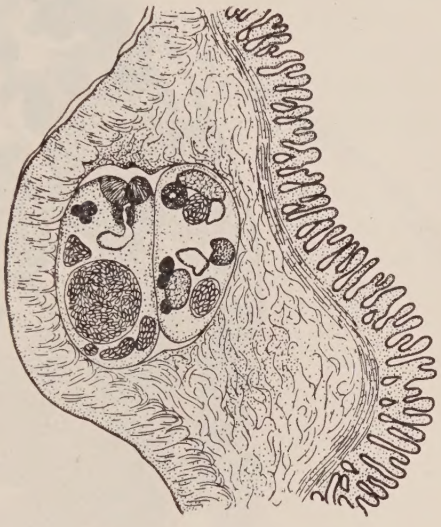

Fig. 4. Section of stomach wall of frog with cyst containing 2 specimens of *Prosotocus mirabilis* sp. n.

Prosotocus mirabilis sp. n.

In contradistinction to *Pl. intermedius* these flukes were found in cysts of connective tissue exclusively in the stomach wall of *Rana terrestris* between the layers of circular and longitudinal muscles (Fig. 4). There were 8 flukes in a cyst and the highest number of flukes found in a frog amounted to 22 specimens.

These flukes are fairly large, red-brownish in colour, their cuticle is densely covered with large spines all over (Fig. 5). A well-developed oral sucker lies subterminally and the ventral sucker lies in the middle of te body or somewhat behind. The structure of the ventral sucker shows a far-advanced atrophy. There is no basin-like hollowing as clearly shown by cross-sections of the flukes. It is no longer likely to perform any adhesive functions.

The alimentary tract. The prepharynx is very small and occurs only in the form of small pockets at the sides of the pharynx. The

pharynx is small and the oesophagus very short. The intestinal branches are relatively long, as they often end at the level of the bottom margin of the ventral sucker.

Fig. 5. *Prosotocus mirabilis* sp. n.

The excretory system. Excretory vesicle, large, V-shaped.

The genital system. Two testes rather irregular and often semilunar in shape lie in front of the intestinal branches. A large circus sac, 0.54—0.66 × 0.16—0.19 mm reaches from the ventral sucker to the front margin of the left testis. Inside there is a small coiled seminal vesicle, a large ejaculatory duct and a pars prostatica. The cirrus sac communicates with the atrium genitale through a narrow canal 0.034—0.043 mm long and 0.003—0.006 mm in diameter. A strongly lobular ovary lies on the rigth side in front of the ventral sucker at the bifurcation of the intestine (Fig. 6). Near the ovary and closer to the dorsal surface there is a small seminal receptacle. Close by runs Laurer's canal; first hardly visible, it opens out on the dorsal side at

the height of the ventral sucker. Tree-like vitelline glands lie between the testes and the oral sucker. The uterus forms two main loops which fill up the hind part of the body. The transverse loop is strongly reduced near to the testes. Only in several flukes an under-developed loop was observed which reached the front margin of the right testis and did not go behind the oesophagus. In most flukes the uterus reaches only the hind margin of the testis. As in the previous species the uterus in older flukes forms sacciform enlargements filled up with a multitude of eggs.

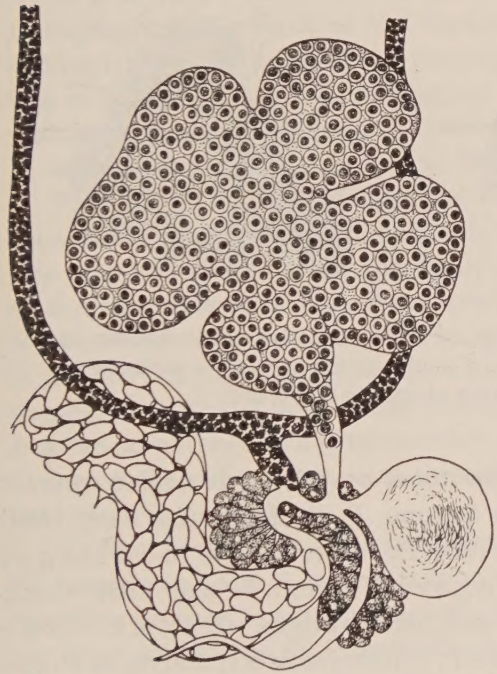

Fig. 6. Structure of female genital organs of *Prosotocus mirabilis* sp. n.

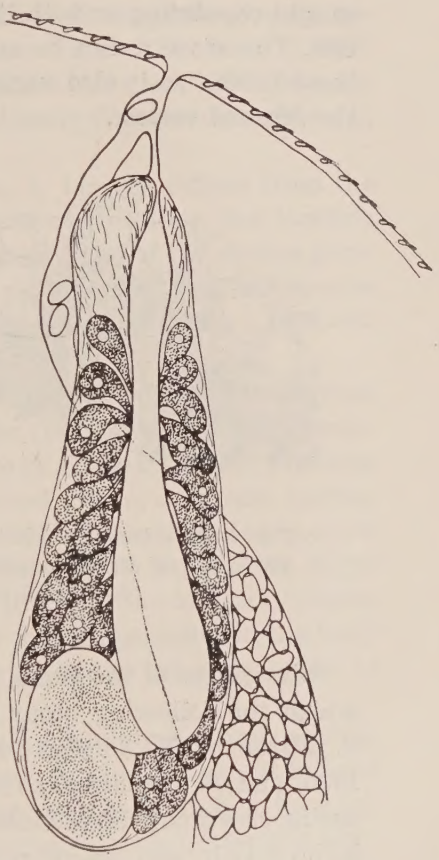

Fig. 7. Structure of copulatory organs in *Prosotocus mirabilis* sp. n. Reconstruction.

The metraterm is very poorly developed, has no distinct muscles and empties into the atrium genitale. Very numerous dark-brown eggs contain fully developed miracidia. The atrium genitale is fairly specifically developed and has undergone a far-going atrophy

(Fig. 7 & 8). It lies on the left border of the body, at the height of the pharynx. Strictly speaking, it is an extention of te metraterm, 0.034 — 0.051 mm long and 0.008 — 0.018 mm wide. The pore leading outside is so small that it is quite invisible on live specimens and total preparations. Because of its size and the occurrence of a very small canal joining the cirrus sac with the atrium, it seems impossible for these flukes to copulate. None of the 64 specimens of this species were caught copulating or with its cirrus extruded as in the previous species. Therefore it can be supposed that autoinsemination occurs in these flukes, as is also suggested by the presence of spermatozoa in the seminal vesicle.

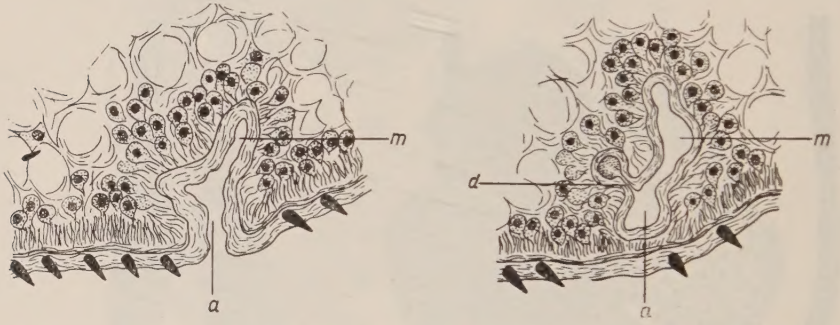

Fig. 8. *Prosotocus mirabilis* sp. n. Two successive cross-sections at the height of atrium genitale. a — atrium genitale, m — metraterm, d — canal joining atrium to cirrus sac.

Six species of the genus *Prosotocus* have been described so far of which the following occur in Europe: *P. confusus* (Looss, 1894), *P. fülleborni* Travassos, 1930, *P. vespertilionis* Mödlinger, 1930, and *P. sigalasi* Baillenger et Chanseau, 1954. Considering very small differences a number of authors (Dawes, Skarbilowich and others) regard *P. fülleborni* as a synonym of *P. confusus*, Odening treats *P. fülleborni* and *P. sigalasi* as subspecies of *P. confusus*. We also think it more probable that *P. vespertilionis* described by Mödlinger from a single specimen found in *Myotis myotis* intestine is a stray parasite and should rather be assigned to *P. fülleborni*, if the latter is regarded as a separate species. Still, the question of ordering the European species of *Prosotocus* remains open and calls for more accurate investigation.

On the other hand, the species regarded by Dawes and after him by Skarbilowich as *Prosotocus amphoraeformis* Mödlinger, 1930, *P. mehelyi* Mödlinger, 1930 and *P. trigonostomum* Mödlinger, 1930 have been assigned to this genus by mistake. Mödlinger describes them as *Distomum amphoraeformis, D. mehelyi, D. trigonostomum,* without describing their taxonomical position more closely. In our opinion these species do belong to the family *Lecithodendriidae* but to other subfamilies. *D. amphoraeformis* belongs to the subfamily *Alassogonoporinae* Skarbilowich, 1947, *D. mehelyi* = *Ophiosacculus mehelyi* (Mödlinger, 1930) Macy, 1935, and *D. trigonostomum* to the subfamily *Gyrabascinae* Macy, 1935.

In structure *Prosotocus mirabilis* sp. n. largely differs from the European species. Lack of the transverse uterine loop, the location of the cirrus sac in front of the ventral sucker and of the atrium genitale in front of the testes, all suggest a closer affinity of this species with those occurring in India: *P. indicus* Mehra et Negi, 1928 and *P. infrequentum* Srivastava, 1933.

The species *Pl. intermedius* and *P. mirabilis,* which we described constitute an interesting exception in the subfamily *Pleurogenetinae.* The representatives of this subfamily are as a rule intestinal parasites of the amphibians. As has been mentioned above, only one species, *Mehraorchis ranarum* Srivastava, 1934, occurring also in cysts in the body cavity of frog, has been known so far. A supposition might suggest itself that flukes found outside the intestine are stray forms. But the occurrence of a great number of mature parasites in the body cavity of frog and absence of these forms in the intestine seems to exclude this supposition.

No less interesting is the simultaneous occurrence of both species, *Pl. intermedius* and *P. mirabilis* in so small an area as Łuże. It follows from the present descriptions of the frog parasitofauna that they are very rare species. As *Pl. intermedius* occurs in Siberia in the frog intestine and *P. mirabilis* is more similar to the species occurring in India, it may be supposed that they are Asiatic species and their occurrence is insular in character. Their occurrence in isolation, conditions differing largely from those of their normal occurrence, may be the reason for the change in their localization in the host.

Author's address:
Zakład Parazytologii PAN
Warszawa, Pasteura 3

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* After Skarbilovich (Trematody żiwotnych i czełowieka. Red. K. J. Skrjabin. II, 1948).

STRESZCZENIE

W trakcie badań nad przywrami żab, prowadzonych w latach 1954—57 na łące Łuże w Puszczy Kampinoskiej, autorka znalazła dwa gatunki przywr zwracających uwagę swoją lokalizacją poza jelitem w torbielach łącznotkankowych. Przywry te zostały określone jako *Pleurogenes intermedius* Isaitchikoff, 1926 i *Prosotocus mirabilis* sp. n.

P. intermedius po raz pierwszy został opisany przez Isaiczikowa z jelita *Rana arvalis altaica* z okolicy Omska na Syberii. Łuże jest drugim z kolei stanowiskiem tego gatunku. Na Łużu autorka stwierdziła występowanie *P. intermedius* wyłącznie w torbielach łącznotkankowych w okolicy pęcherza moczowego u 31,6% *Rana terrestris* i jednej *R. temporaria*.

W porównaniu z formami jelitowymi opisanymi przez Isaiczikowa przywry występujące na Łużu są większe, ciało ich ma kształt wałeczkowaty oraz są znacznie mniej ruchliwe. Prawdopodobnie jest to związane ze zmianą lokalizacji pasożyta.

Prosotocus mirabilis sp. n. jest gatunkiem rzadkim. Autorka znalazła go jedynie u 3,3% *Rana terrestris* w torbielach łącznotkankowych w ściance żołądka, między warstwą mięśni okrężnych i podłużnych. U gatunku tego występuje daleko posunięta atrofia przyssawki brzusznej i atrium genitale. Budowa atrium nasuwa przypuszczenie, że kopulacja u tych przywr nie występuje, a rozród odbywa się drogą samozapłodnienia, o czym świadczy obecność plemników w receptaculum seminis.

P. mirabilis sp. n. różni się dość znacznie od europejskich gatunków rodzaju *Prosotocus*. Brak poprzecznej pętli macicy w okolicy jąder, położenie torebki prąciowej przed przyssawką brzuszną i atrium genitale przed lewym jądrem wskazują na bliższe pokrewieństwo tego gatunku z występującymi w Indiach *P. indicus* Mehra et Negi, 1928 i *P. infrequentum* Srivastava, 1933.

Zdaniem autorki oba gatunki znalezione na Łużu są gatunkami azjatyckimi, przy czym ich występowanie ma prawdopodobnie charakter wyspowy. Zmiana lokalizacji w żywicielu może być spowodowana występowaniem w warunkach izolacji, różniących się znacznie od warunków, w jakich te przywry normalnie występują.